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(54) Testing for the presence of living organisms at the surface of an object

(57) A method of determining the quantity of living organisms at the surface of an object involves applying a bioluminescent agent, for example firefly luciferin-luciferase enzyme, onto the surface, conveniently by spraying. NRB nucleotide releasing reagent is then sprayed onto the surface wetted with the enzyme solution. As the ATP-bearing cells of any micro organisms present at the surface of the sample are disrupted by the releasing agent, the ATP is released into an environment of firefly enzyme whereby bioluminescence is emitted in the immediate vicinity of the cell from which the ATP was released.

The luminscence is then monitored by apparatus which has a display device which may be calibrated to indicate the level of the emitted luminescence, or where a particular organism is to be detected directly in quantity of organism per unit area.

GB 2 178 847 A

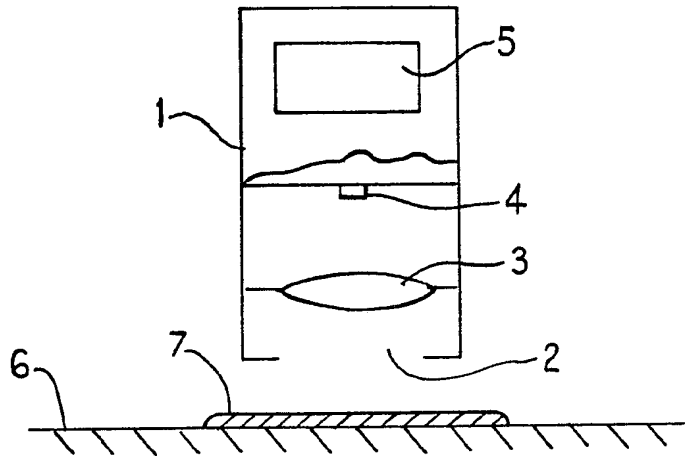

FIG.1

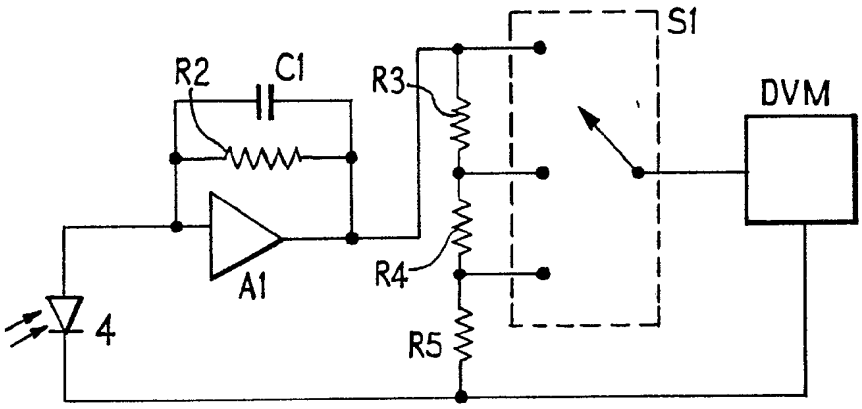

FIG.2

SPECIFICATION

Testing for the presence of living organisms at the surface of an object

The invention relates to a method of determining the quantity of living organisms at the surface of an object, the living organisms having cells bearing a biochemical substance which is capable of reaction with a specific enzyme to produce bioluminescence comprising the steps of applying to the surface an agent for the release of the biochemical substance from the cells, providing a bioluminescent agent for reaction with the released biochemical substance to produce bioluminescence and detecting the luminescence produced. The invention further relates to apparatus for carrying out the method.

It has been known for several years that all living organisms contain adenosine triphosphate (ATP), and that hydrolytic reaction of ATP with certain enzymes results in the production of light, although the mechanism of the reaction is not necessarily fully understood. The amount of light, referred to as bioluminescence, emitted is very small, and it has been the practice, in laboratory experiments with the reaction, to determine the light emission by use of a photometer.

The ATP is contained in cells within the organisms, and can be released from the cells by the application of a reagent capable of attacking the cell membrance. It has now been realised that the reaction can be adapted to provide a simple, rapid and essentially non-destructive test for the contamination by micro-organisms of the surface of objects such as pieces of textile fabric.

ATP is, in fact, only one of a group of biochemical substances, each of which is capable of reaction with a specific enzyme to produce bioluminescence, and although in the following specification reference will be made for the sake of simplicity primarily to ATP, it should be understood that the term is to be construed as extending to any member of the aforementioned group or a derivative thereof.

U.K. Patent Application No. 2116709A describes and claims a method of test for the presence of living organisms having ATP-bearing cells at the surface of an object, comprising applying to the surface an agent for the release of ATP from the cells, providing a bioluminescent agent for reaction with released ATP to produce bioluminescence, and observing the luminescence produced.

That application suggests that the luminescence can be seen by the eye or recorded by a camera with the aid of an image intensifier. It also suggests that by providing the image intensifier with a video camera and recording system, and provided that the reagents applied do not inhibit the growth of the micro organisms, the technique may provide a method for observing and recording the developing extent of contamination as a function of time. However, image intensifiers are normally operated to give a constant output brightness once a threshold image brightness has been exceeded and consequently a determination of the quantity of organisms at a given point is not feasible by viewing the bioluminescence through the image intensifier or by recording the image by means of a camera.

It is an object of the invention to enable a quantitative determination of the presence of living organisms at the surface of an object.

The invention provides a method of determining the quantity of living organisms at the surface of an object as set forth in the opening paragraph characterised in that the luminescence is monitored by a detection device which produces an electrical output which is dependent on the intensity of the luminescence falling on the detection device, that the electrical output is converted to a measure of the quantity of the living organisms present, and that the converted output is arranged to drive a display device.

The invention further provides apparatus arranged to carry out the method set forth in the preceding paragraph, the apparatus comprising a detection device for receiving the luminescence and converting the received luminescence to an electrical signal whose magnitude is dependent on the intensity of the received luminescence, means for converting the electrical signal to a signal which is representative of the quantity of living organisms present in a given area, and means for displaying the converted signal.

The method and apparatus set forth in the two preceding paragraphs have the advantage over the method disclosed in U.K. Patent Application No. 2116707A that a quantitative determination of the living organisms present at the surface of an object can be conveniently made. Although that application makes reference to semi-quantitative determination by estimation of the luminescence seen through an image intensifier this is only feasible over a limited range of intensities since the image intensifier will either saturate or have its gain reduced by an automatic gain control arrangement once a threshold value has been reached. Another semi-quantatitive approach suggested by the cited application is to repeatedly photograph the image intensifier output over a period of time and thus record the change in area of contamination. This is a time consuming task and will only indicate whether the area of contamination is changing and will not give a reliable indication of the degree of contamination at a particular point.

The detection device may comprise a photo-diode or a photo-transistor. Alternatively the detection device may comprise a photomultiplier tube.

These alternatives allow a comparatively inexpensive monitoring apparatus to be constructed and in particular when a photo-diode or photo-transistor is used a portable battery operated apparatus may be constructed. There are other devices which may be used as the detection device, for example the electrical output of the

automatic gain control circuit of an image intensifier could be used provided that the lower detection limit, which is determined by the threshold illumination at which the automatic gain circuit starts to operate, is sufficiently low for the required sensitivity of detection.

In order to concentrate the maximum illumination on the detection device a lens or mirror may be provided for focussing the received luminescence on the detection device. This will increase the detection sensitivity of the apparatus.

The display means may comprise a digital voltmeter. This can be a comparatively compact and inexpensive item enabling a digital display of the quantity of organisms present.

An embodiment of the invention will now be described, by way of example, with reference to the accompanying drawings, in which:-

Figure 1 shows in partial cross section apparatus for carrying out the method of determining the quantity of living organisms at the surface of an object; and

Figure 2 shows a circuit diagram of the apparatus of Figure 1. A method according to the inventin of determining the quantity of living organisms at the surface of an object involves applying a bioluminescent agent, for example Firefly luciferin-luciferase enzyme, onto the surface, conveniently by spraying. No significant reaction with ATP from surface contamination occurs at this stage because the ATP is predominantly enclosed within cell membrances. Commercially available firefly enzyme may itself be slightly contaminated with ATP however and there may be a slight bioluminescent background from reaction of the enzyme with such traces of ATP, and such background luminescence should be discounted in considering that revealed after the next subsequent step.

NRB nucleotide releasing reagent is then sprayed onto the surface wetted with the enzyme solution. As the ATP-bearing cells of any micro organisms present at the surface of the sample are disrupted by the releasing agent, the ATP is released into an environment of firefly enzyme whereby bioluminescence is emitted in the immediate vicinity of the cell from which the ATP was released.

For instruction on the preparation of the Firefly luciferin-luciferase enzyme reference should be made to the aforementioned patent application.

The luminscence is then monitored by apparatus which is described hereinafter to determine the level of the emitted bioluminescence. The apparatus has a display device which may be calibrated to indicate the level of the emitted luminescence, or where a particular organism is to be detected directly in quantity of organism per unit area.

Figure 1 shows in partial cross-section apparatus for carrying out the method described. An ashown in Figure 1 the apparatus comprises a body 1 of any arbitrary cross-section, for example circular or rectangular, the body 1 having an opening 2 through which light can pass to a lens 3 mounted within the body 1. The lens 3 is arranged to focus the light passing through the opening 2 onto a detector 4, which may be a photo-diode. The detector is connected in an electrical circuit such as that shown in Figure 2. A display device 5 is mounted in the body 1 to provide an indication of the quantity of organisms present in an area corresponding to the area of the opening 2. To perform the method the ATP and NRB nucleotide releasing reagent are sprayed on the surface 6 of an object to be investigated and the organisms 7 then luminesce. The body 1 is then placed over the luminescing organisms so that the emitted radiation passes through the opening 2 and is focussed on the detector 4. A reading is then produced on the display device 5. It is, of course, important to exclude ambient light from the detector 4 and thus the opening 2 must be placed in contact with the surface of the object.

Figure 2 shows the detector 4 in the form of a photodiode which is connected to the input of an operational amplifier A1. The parallel arrangement of a resistor R2 and a capacitor C1 is connected between the input and output of the amplifier A1. The series arrangement of three resistors R3, R4 and R5 is connected between the output of the amplifier A1 and the terminal 11. A selection switch S1 has three selection contacts which are connected to the output of the amplifier A1, the junction of resistors R3 and R4 and the junction of resistors R4 and R5, respectively. The pole of the switch S1 is connected to the input of a digital voltmeter DVM the display of which forms the display device 5.

Thus in this embodiment the photodiode 4 forms the detection device, the operational amplifier and digital voltmeter form the conversion means, and the display on the digital voltmeter forms the means for displaying the converted signal. Clearly the conversion means may comprise further signal processing circuitry to linearise the response, to determine the measurement time, and to reduce the effects of noise. Such signal processing circuitry would be well known to those skilled in the art.

A photo-diode or photo-transistor form convenient detection devices since they enable a low voltage power supply to be used enabling a compact battery operated unit to be constructed. However if greater sensitivity is required they would be replaced by a photomultiplier tube or an image intensifier as discussed hereinbefore. The lens 3 may be omitted if the detector 4 is sufficiently sensitive. Since the purpose of the lens 3 is to converge the emitted luminescence on the detector it could be replaced by other optical elements such as a mirror.

The method may be used for the monitoring of microbiological contamination of a textile fabric, for example mildew on woollen cloth, or for contamination of other surfaces, for example in hospital operating threatres, and can be carried out simply without handling of the objects.

CLAIMS

1. A method of determining the quantity of living organisms at the surface of an object, the living organisms having cells bearing a biochemical substance which is capable of reaction with a specific enzyme to produce bioluminescence comprising the steps of applying the surface an agent for the release of the biochemical substance from the cells, providing a bioluminescent agent for reaction with the released biochemical substance to produce bioluminescence and detecting the luminescence produced characterised in that the luminance is monitored by a detection device which produces an electrical output which is dependent on the intensity of the luminescence falling on the detection device, that the electrical output is converted to a measure of the quantity of the living organisms present in a given area, and that the converted output is arranged to drive a display device.

2. A method as claimed in Claim 1, characterised in that the detection device comprises a photo-diode or a photo-transistor.

3. A method as claimed in Claim 1, characterised in that the detection device comprises a photomultiplier tube.

4. A method as claimed in any preceding claim, characterised in that the luminescence is focussed on the detection device by a lens.

5. A method of determining the quantity of living organisms at the surface of an object substantially as described herein with reference to the accompanying drawings.

6. Apparatus arranged to carry out the method of Claim 1, the apparatus comprising a detection device for receiving the luminescence and converting the received luminescence to an electrical signal whose magnitude is dependent on the intensity of the received luminescence, means for converting the electrical signal to a signal which is representative of the quantity of living organisms present in a given area, and means for displaying the converted signal.

7. Apparatus as claimed in Claim 6, in which the detection device comprises a photo-diode or a photo-transistor.

8. Apparatus as claimed in Claim 6, in which the detection device comprises a photomultiplier tube.

9. Apparatus as claimed in any of Claims 6 to 8, comprising means for focussing the received luminescence on the detection device.

10. Apparatus as claimed in Claim 9, in which the focussing means comprises a lens.

11. Apparatus as claimed in any of Claims 6 to 10, in which the display means comprises a digital voltmeter.

12. Apparatus arranged to carry out the method of Claim 1, the apparatus being substantially as described herein with reference to the accompanying drawings.

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